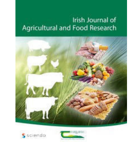

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Yield of binary- and multi-species swards relative to single-species swards in intensive silage systems

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Abstract

Binary- and multi-species sown mixtures may increase herbage yield and/or reduce inorganic nitrogen (N) requirement compared to perennial ryegrass (PRG) (Lolium perenne L.) swards. A split-plot design was used to compare yields of binary- and multi-species mixtures to single-species swards of three grasses and red clover managed for intensive silage production under varying N application rates. Perennial and Italian (Lolium multiflorum Lam.) ryegrasses had greater annual yields when grown as single species receiving 360 kg N/ha per year than in binary mixtures with red clover (Trifolium pratense L.) receiving 0 kg N/ha per year, whereas timothy (Phleum pratense L.) produced equally high yields in both situations. When no inorganic N was applied, the annual dry matter yield of Mix 1 (10,738 kg/ha; PRG, timothy, red clover and white clover (Trifolium repens L.) and Mix 2 (11,679 kg/ha; PRG, timothy, red clover, ribwort plantain (Plantago lanceolata L.) and chicory (Cichorium intybus L.)) was greater than that of a PRG sward (PRG/0N; 5,885 kg/ha) and derived more from the contribution of legumes than herbs. This yield advantage of mixtures declined as inorganic N input increased, as did the legume and herb proportions in the multi-species swards. When averaged across rates of inorganic N input, Mix 2 had a greater annual yield than Mix 1 (12,464 vs. 11,893 kg/ha). Mix 2 receiving no inorganic fertiliser N and both Mix 1 and Mix 2 receiving 120 kg N/ha per year matched the annual yield achieved by PRG receiving 360 kg N/ha per year. Our results indicate that the yield performance of binary- and multi-species grassland swards should be measured in situ rather than predicted from single-species swards of constituent species.

Keywords

Dry matter yield • grass-clover swards • multi-species swards • nitrogen response • silage

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most commonly sown forage species in temperate grassland-based ruminant production systems due to its potential for high yields of good quality herbage with strong persistence under both grazing and cutting management systems (Frame and Laidlaw, 2011). However, to fulfil its production potential, a perennial ryegrass sward requires fertile soil and high rates of inorganic nitrogen (N) input (Frame, 1991). The sustainability of these high input, intensively managed single-species systems becomes questionable if the cost of inorganic N increases and/or if negative environmental impacts due to its application increase (Galloway *et al.*, 2008; Canfield *et al.*, 2010). At the same time, the requirement from governments and wider society for farmers to increase livestock production in a sustainable manner, i.e. produce more food from the same area of land while reducing any negative environmental impacts, is likely to continue and become more intense (Reheul *et al.*, 2017).
Compared to perennial ryegrass swards, binary- and multi-species swards can maintain herbage yields at reduced inorganic N fertiliser inputs or increase herbage yields at similarly high inorganic N inputs (Connolly *et al.*, 2009; Nyfeler *et al.*, 2009; Suter *et al.*, 2015). These benefits have been attributed to complementary interspecific interactions such as biological N fixation by rhizobia within the root nodules of legumes (Nyfeler *et al.*, 2011) as well as spatial and temporal resource-use efficiency that can occur as functional diversity increases (Cardinale *et al.*, 2007; Schmid *et al.*, 2008).
Within grassland-based ruminant production systems, silage can range from an opportunistically produced feed in a grazing-dominant system to the primary forage in higher input systems where animals are housed for extended durations (O'Kiely and Muck, 1998). Although information is available on the yield of multi-species swards compared to single-species swards and simpler mixtures under cutting (Lüscher *et al.*, 2008; Finn *et al.*, 2013; Lüscher *et al.*, 2014) and grazing regimes (Pembleton *et al.*, 2015; Roca-Fernández *et al.*, 2016; Vibart *et al.*, 2016),

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there is a shortage of information on how they relate to the annual and individual cut yield of grass swards and grass–legume binary mixtures of some of the constituent species when managed under an annual four-cut, silage production regime. Furthermore, there is also a deficit of information for such circumstances on how incremental inputs of inorganic N impact on the yield and persistence of multi-species swards compared to their effects on perennial ryegrass swards (Pembleton *et al.*, 2015). These factors can have a marked impact on farm system efficiency and profitability.

The objectives of this study were to quantify the effects on herbage yield of (1) three common temperate grass species receiving inorganic N or grown in binary mixture with red clover, (2) a perennial ryegrass sward *versus* either a perennial ryegrass plus red clover binary mixture or two multi-species swards, each grown with zero inorganic N input and (3) the response of a perennial ryegrass sward *versus* two multi-species mixtures to increasing rates of inorganic N in Years 2 and 3 post sowing. Since the timing of the harvest of the primary growth of herbage can have an important impact on the yield of both the first and second cuts for silage production (Gilliland *et al.*, 1995; King *et al.*, 2012) and since these impacts may differ with sward type, the effects of harvest schedule (specifically the timing of the primary growth harvest) were also investigated. The latter would also allow elucidation of the rates of change in yield at the time when the primary growth is harvested. Finally, sward botanical composition was monitored to assess if any early indications of treatment effects on sward persistence could be gleaned.

Materials and methods

Field plots

Field plots (each 10 m ´ 2 m) were established in September 2012 at Teagasc, Grange (53.52°N, 6.66°W, 85 m above sea level) on a brown earth soil composed of imperfectly drained clay loam with a high base status (Gardner, 1962). The preceding permanent grassland sward was sprayed with a broad-spectrum herbicide (360 g glyphosate/L applied at 6 L/ha; Monsanto Europe) before being ploughed and cultivated to produce a fine tilth. Based on analysis of soil samples, inorganic P and K fertiliser was applied to raise soil concentrations to index 3 (Coulter *et al.*, 2008) and lime was applied to raise the pH to a target 6.3.

In each of four replicate blocks, treatments were allocated in a split-plot design. The main plots involved the primary growth harvest of a four cut per year schedule being on either 12–13 May (Early), 26–27 May (Middle) or 9–10 June (Late). The subplots involved 18 treatments differing in herbage species and inorganic N input, with each species within a treatment represented by an equal weight of seed from three cultivars (two for chicory and one for ribwort plantain) at sowing (Tables 1 and 2). Seed was manually sown on 5–6 September 2012, and all plots were tactically spot sprayed with a herbicide (containing 2,4 Dichlorophenoxyacetic sodium salt, 2-methyl-4-chlorophenoxyacetic acid (MCPA) potassium salt, benazolin potassium salt, trisodium nitrilotriacetate and potassium hydroxide; Legumex Extra; Aventis CropScience UK Ltd.) in April 2013. During 2013, plots were fertilised and harvested as per the two following experimental years, but no data recording took place.

Plots were managed in a silage production regime with the annual yield of biomass being harvested in four successive cuts during 2014 (Year 1) and 2015 (Year 2). The primary growth harvest (Cut 1) for the Early, Middle and Late harvest schedules were on 13 May 2014, 27 May 2014 and 10 June 2014 in Year 1, and 12 May 2015, 26 May 2015 and 9 June 2015 in Year 2. Cuts 2 and 3 were harvested 7 and 14 weeks after their primary growth harvest, while Cut 4 in all cases was on 10 and 24 November of Years 1 and 2, respectively. Inorganic N was applied as calcium ammonium nitrate (275 g N/kg) with 0.333, 0.278, 0.222 and 0.167 of the annual allocation being applied to coincide with the beginning of the growth of herbage to be harvested at Cuts 1–4, respectively. Therefore, inorganic N was applied in mid-March and immediately after Cuts 1–3. Simultaneously, a compound fertiliser containing 70 g P and 300 g K/kg was applied at 400 kg/ha for Cut 1 and 143 kg/ha for each of Cuts 2–4 (i.e. 58 kg P and 249 kg K/ha per year).

At each cut, herbage was harvested to an approximate stubble height of 6 cm and weighed using a Haldrup forage plot harvester (J. Haldrup, Løgstør, Denmark) before being chopped by a precision-chop forage harvester (MEX V1; Pottinger, Grieskirchen, Austria). Chopped herbage was sampled (*ca*. 2 kg) and stored at -18°C prior to determination of dry matter (DM) content. Herbage DM content was estimated following drying in a forced-air circulation oven at 98°C for 16 hours.

The proportion of biomass contributed by each species in each plot was visually estimated as described by Clavin *et al.* (2017) and recorded as a percentage of the total herbage within each plot. This estimate was determined by the same trained assessor throughout the study.

Meteorological data were recorded within 1 km of the field plots, and rainfall, air temperature, solar radiation, relative humidity, soil temperature and evapotranspiration potential values are summarised in Figure 1.

Figure 1. Mean daily meteorological results shown at weekly intervals for (a) rainfall (mm/day), (b) air temperature (°C), (c) solar radiation (J/cm²), (d) relative humidity (%), (e) soil temperature at 10 cm depth (°C) and (f) potential evapotranspiration rate (mm/day). The harvests for Cuts 1, 2 and 3 in both years were during weeks 20–24, 27–31 and

Table 1. Species, cultivars and standard seeding rates

Species	Cultivar[1]	Standard rate[2]
TIM (*Phleum pratense* L.)	Comer (9/6; H), Erecta (10/6; H), Promesse (10/6; H)	15
IRG (*Lolium multiflorum* Lam.)	Fabio (19/5; T), Nabucco (21/5; T), Davinci (23/5; D)	42
PRG (*Lolium perenne* L.)	Premium (23/5; D), Shandon (21/5; D), Solomon (22/5; D)	32
RC (*Trifolium pratense* L.)	AberRuby (D), Amos (T), Merviot (D) (all early flowering)	15
White clover (*Trifolium repens* L.)	Aran (v.large leaf), Barblanca (large leaf), Chieftain (medium leaf)	12
Ribwort plantain (*Plantago lanceolata* L.)	Ceres Tonic (D)	12
Chicory (*Cichorium intybus* L.)	Grasslands Choice (D), Puna (D)	5

[1]Heading date (day/month), ploidy (D – diploid, T – tetraploid, H – hexaploid) and other cultivar classification characteristics.
[2]Standard rate of seed used if sown as a monoculture (kg seed/ha).
TIM = Timothy, IRG = Italian ryegrass, PRG = perennial ryegrass, RC = red clover.

Table 2. Sward types and the associated species included, rates of seed used and rates of inorganic N applied

Sward	Species included	Seed rate[1]	N[2]
TIM/360N	Timothy	100	360
IRG/360N	Italian ryegrass	100	360
PRG/0-360N	Perennial ryegrass	100	0, 120, 240, 360
RC	Red clover	100	0
TIM/RC	Timothy, red clover	40, 60	0
IRG/RC	Italian ryegrass, red clover	40, 60	0
PRG/RC	Perennial ryegrass, red clover	40, 60	0
Mix 1/0-360	Timothy, perennial ryegrass, red clover, white clover	20, 20, 35, 25	0, 120, 240, 360
Mix 2/0-360	Timothy, perennial ryegrass, red clover, ribwort plantain, chicory	20, 20, 35, 12.5, 12.5	0, 120, 240, 360

[1]Rate of seed used as a percentage of its standard monoculture rate shown in Table 1 (values correspond in order with species in the preceding column).
[2]Inorganic fertiliser N input (kg N/ha per year).

34–38, respectively, while Cut 4 was in weeks 46 and 48 of Years 1 and 2, respectively. Week refers to week of the calendar year.

Statistical analysis

The 18 subplot treatments within the main plots (three levels of harvest schedule) of this split-plot design had a number of subsets with factorial structure and associated controls. The nested model, or elaborate contrasts, required to incorporate the controls and the multiple factorial sets of treatments resulted in undue complexity, and to avoid this, the subplot treatments were arranged into three groups (Groups 1–3) for statistical analysis. The treatment contrasts within these groups addressed the three objectives identified at the end of the Introduction.

Group 1 used seven subplot treatments (PRG/360N, IRG/360N, TIM/360N, PRG/RC, IRG/RC, TIM/RC and RC) to give a (3 ´ 2) + 1 arrangement, with the +1 (i.e. RC) being a control. A nested linear model was used to accommodate this structure. For Group 2, the four subplot treatments PRG/0N, PRG/RC, Mix 1/0N and Mix 2/0N formed a simple four treatment contrast, and in Group 3, 12 subplot treatments (PRG/0, 120, 240 or 360N, Mix 1/0, 120, 240 or 360N and Mix 2/0, 120, 240 or 360N) provided a 3 ´ 4 factorial arrangement. All analyses of these groups incorporated year, replicate blocks and harvest schedule as the main plot factor. In the first instance, harvest schedule was included in analyses as a factor, and then, with equally spaced time intervals, the analyses were repeated with schedule as a covariate in an analysis of covariance to identify trends over time. All interactions were tested, and in the analysis of covariance, linear and quadratic terms and their interactions were included. A similar analysis of covariance was used for nitrogen rates in Group 3. Within each of Groups 1–3, analyses for annual yield were performed using the cumulative values from four cuts.

Residuals from all analysis models were checked to ensure that the assumptions of the analyses were met. Contrasts

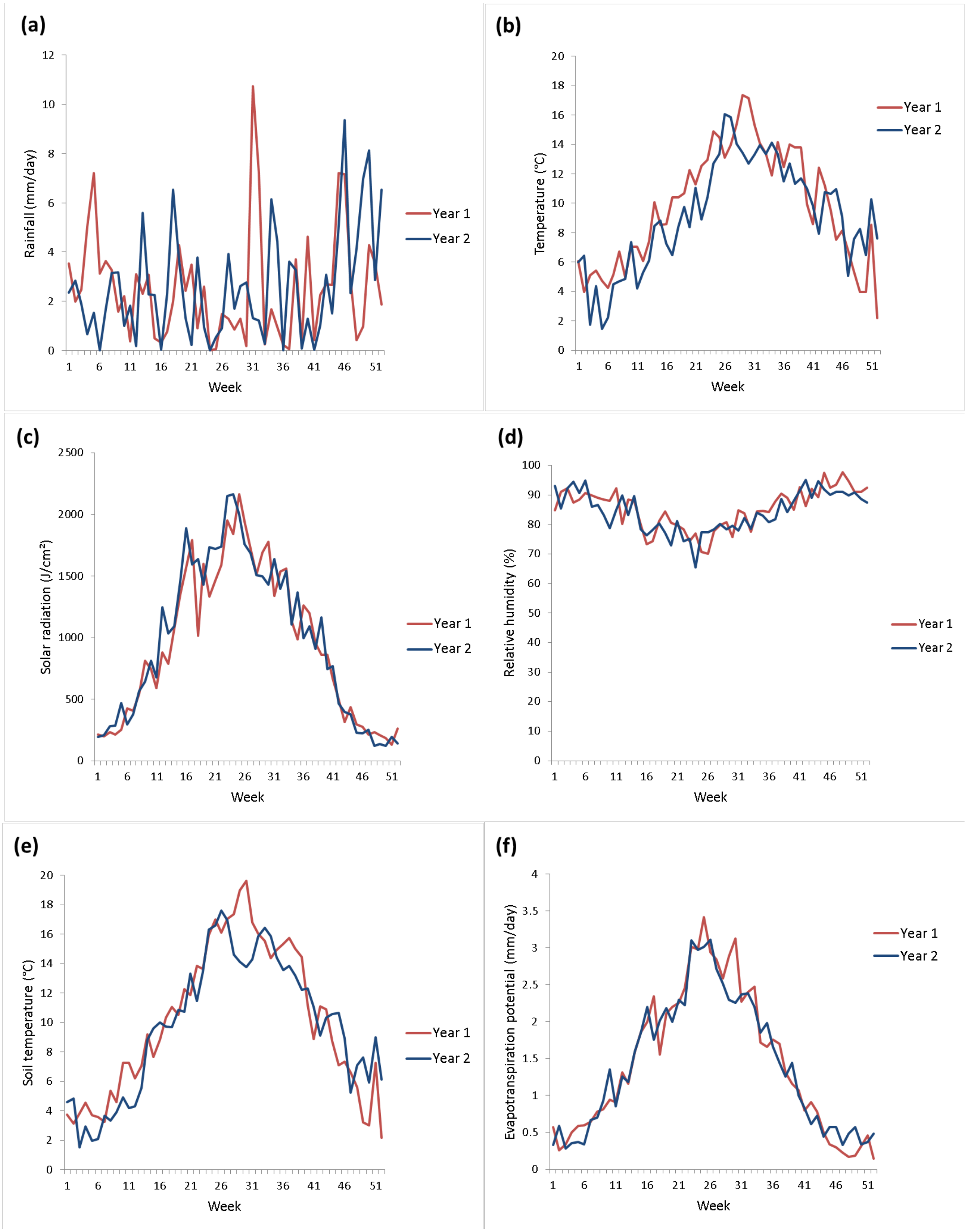

Figure 1. Mean daily meteorological results shown at weekly intervals for (a) rainfall (mm/day), (b) air temperature (°C), (c) solar radiation (J/cm²), (d) relative humidity (%), (e) soil temperature at 10 cm depth (°C) and (f) potential evapotranspiration rate (mm/day). The harvests for Cuts 1, 2 and 3 in both years were during weeks 20–24, 27–31 and 34–38, respectively, while Cut 4 was in weeks 46 and 48 of Years 1 and 2, respectively. Week refers to week of the calendar year.

Table 3. Mean DM yield (kg/ha) at each cut and annually for each harvest schedule and sward species ´ inorganic N treatment (averaged across 2 years)

Cut	1			2			3			4			Annual[3]
Schedule[1]	E	M	L	E	M	L	E	M	L	E	M	L	
Species[2]													
IRG/360N	5,961	6,001	6,048	4,121	3,169	2,588	2,248	1,977	2,287	2,345	1,822	1,120	13,229
TIM/360N	6,029	5,572	6,712	2,671	2,525	2,020	2,042	2,164	2,124	1,788	1,405	1,231	12,095
RC	3,058	4,869	5,295	3,944	3,658	4,181	3,499	2,728	1,796	800	590	1,101	11,840
IRG/RC	4,871	5,084	5,132	2,725	2,276	2,317	1,775	1,227	1,052	1,656	1,208	753	10,025
TIM/RC	4,054	5,581	6,181	3,619	3,113	2,941	2,978	2,085	1,734	1,182	698	1,208	11,791
PRG/RC	3,732	5,120	5,906	3,090	2,422	2,403	3,162	1,811	1,288	1,452	936	993	10,771
PRG/0N	3,712	4,274	3,936	1,013	734	730	453	509	651	719	481	444	5,885
PRG/120N	4,687	5,312	5,960	2,582	1,536	1,461	1,376	983	1,408	1,327	987	934	9,517
PRG/240N	5,469	5,731	5,852	2,936	2,361	2,040	1,840	1,762	2,029	1,851	1,347	1,114	11,443
PRG/360N	5,721	6,112	6,251	3,164	2,297	1,871	1,886	1,804	2,125	2,097	1,523	1,143	11,998
Mix 1/0N	4,010	4,533	5,796	3,363	2,424	2,142	2,521	2,079	1,661	1,324	1,105	1,257	10,738
Mix 1/120N	5,248	5,708	5,858	3,012	2,379	2,111	2,204	2,057	1,794	1,684	1,330	1,222	11,536
Mix 1/240N	5,245	6,067	6,505	3,464	2,813	2,183	2,510	2,067	2,144	2,002	1,577	1,271	12,616
Mix 1/360N	5,558	6,103	6,402	3,273	2,763	2,205	2,290	2,227	2,293	2,065	1,588	1,280	12,682
Mix 2/0N	3,841	5,558	6,057	3,281	2,730	2,850	2,917	2,044	1,664	1,681	1,324	1,091	11,679
Mix 2/120N	4,728	5,426	6,602	3,335	2,717	2,543	2,636	1,907	1,799	2,191	1,444	1,186	12,171
Mix 2/240N	5,495	6,237	6,581	3,770	2,937	2,451	2,551	2,233	2,141	2,037	1,761	1,228	13,141
Mix 2/360N	5,478	6,200	5,987	3,595	2,767	2,379	2,352	2,186	2,377	2,235	1,668	1,375	12,866

[1]Harvest schedule: E = Early, M = Middle, L = Late.
[2]Sward species × inorganic N treatment.
[3]Annual DM yield averaged across three harvest schedules.
DM = dry matter, IRG = Italian ryegrass, TIM = timothy, RC = red clover, PRG = perennial ryegrass.

between means were specified for significant effects in the analyses, and allowance for multiplicity effects used Tukey adjustments to *P*-values.

All data were analysed using the GLIMMIX and MIXED procedures of SAS 9.4 (SAS, 2013).

Results

The yields for the 18 species ´ N treatments per harvest schedule are presented in Tables 3 (mean values) and 4 (standard errors of the mean and *P*-values). However, since the data were analysed within groups that addressed three specific questions, the following text is organised to reflect this latter structure. Interactions rather than main effects are described where appropriate.

Perennial ryegrass, Italian ryegrass and timothy receiving inorganic N or grown with red clover (PRG/360N, IRG/360N, TIM/360N, PRG/RC, IRG/RC, TIM/RC and RC)

Annual yield

Overall, annual DM yield was greater ($P < 0.001$) when grasses received inorganic fertiliser N compared to when grown with red clover (12,440 vs. 10,862 kg/ha) (Figure 2). Specifically, Italian ryegrass (13,299 kg/ha) yielded greater ($P < 0.001$) than perennial ryegrass (11,998 kg/ha) and timothy (12,095 kg/ha) when receiving 360 kg N/ha (Table 3). However when grown with red clover, timothy (11,791 kg/ha) outyielded both Italian (10,025 kg/ha) and perennial (10,771 kg/ha) ryegrasses. Furthermore, TIM/RC did not differ ($P > 0.05$) from RC (11,840 kg/ha).

Table 4. SEM and P-values for DM yield (kg/ha) at each cut and annually, for each harvest schedule and sward species × inorganic N treatment (averaged across 2 years)

Group[1]		1				2		3			
Effect		Species[2]	N source[2]	Species × source	Species × source × schedule	Species[3]	Species × schedule	Species[4]	N rate	Species × N rate	Species × N rate × schedule
Cut 1	SEM	131.2	114.3	174.2	292.3	158.2	273.9	113.8	122.7	181.1	305.0
	P	0.326	<0.001	0.691	0.079	<0.001	<0.001	<0.001	<0.001	0.006	0.082
Cut 2	SEM	79.9	65.2	113.0	195.7	100.9	174.8	49.8	56.8	94.8	164.1
	P	0.01	0.566	<0.001	0.319	<0.001	0.162	<0.001	<0.001	<0.001	0.334
Cut 3	SEM	64.5	53.6	89.5	155.1	87.7	151.9	49.9	53.0	74.8	126.7
	P	<0.001	0.018	<0.001	0.079	<0.001	<0.001	<0.001	<0.001	<0.001	0.013
Cut 4	SEM	56.5	53.0	65.8	114.0	70.2	121.6	81.7	84.2	101.4	175.6
	P	<0.001	<0.001	0.453	0.46	<0.001	0.032	<0.001	<0.001	<0.001	0.721
Annual	SEM	221.2	189.1	297.3	515.0	246.5	405.3	176.2	188.6	260.6	442.5
	P	0.335	<0.001	<0.001	0.966	<0.001	0.568	<0.001	<0.001	<0.001	0.777

[1]Group 1 = PRG/360N, IRG/360N, TIM/360N, PRG/RC, IRG/RC, TIM/RC and RC (the SEMs for species were calculated for the 3 × 2 interaction but were also used when comparing RC to any of the 3 × 2 treatments); Group 2 = PRG/0N, PRG/RC, Mix 1/0N and Mix 2/0N and Group 3 = PRG/0–360N, Mix 1/0–360N and Mix 2/0–360N.
[2]Within Group 1, species is IRG, PRG or TIM and N source is either grass + 360 kg N/ha per year or grass + red clover.
[3]Within Group 2, species is PRG/0N, PRG/RC, Mix 1/0N and Mix 2/0N.
[4]Within Group 3, species is PRG, Mix 1 and Mix 2. Schedule = harvest schedule.
SEM = standard errors of the mean, DM = dry matter, PRG = perennial ryegrass, IRG = Italian ryegrass, TIM = timothy, RC = red clover.

Figure 2. Annual DM yield of (a) IRG, PRG and TIM grown with 360 kg inorganic nitrogen/ha per year or with RC, and RC grown alone; (b) PRG + red clover (PRG/RC), Mix 1 and Mix 2 receiving 0 kg inorganic nitrogen/ha per year and (c) PRG, Mix 1 and Mix 2 at inorganic nitrogen rates of 0, 120, 240 and 360 kg/ha per year. All values are averaged across the Early, Middle and Late harvest schedules. DM = dry matter, IRG = Italian ryegrass, PRG = perennial ryegrass, TIM = timothy, RC = red clover.

The timing of the primary growth harvest influenced the annual yield of grasses receiving inorganic fertiliser N such that greater ($P < 0.05$) yields were recorded for the Early (12,395 kg/ha) harvest schedule than either the Middle (11,322 kg/ha) or Late (11,238 kg/ha) schedules. However, when grasses were grown in a mixture with red clover, the Late schedule produced a greater ($P < 0.05$) annual yield than the Early schedule (12,373 kg/ha vs. 11,301 kg/ha).

Cuts 1, 2, 3 and 4 yields

At Cut 1, grasses receiving inorganic N yielded greater ($P < 0.001$) than grasses grown with red clover (6,045 vs. 5,073 kg/ha), while the red clover sward had a lower yield ($P < 0.05$) than the other six sward species treatments (Table 3). Delaying the harvest of Cut 1 resulted in a greater ($P < 0.001$) increase in yield when grasses were grown with red clover than when receiving inorganic N. There was a yield increase ($P < 0.05$) when the Cut 1 harvest date of PRG/360N and TIM/360N was delayed but not ($P > 0.05$) of IRG/360N. Thus, IRG/360N (5,416 kg/ha) had a greater ($P < 0.05$) yield than PRG/360N (4,726 kg/ha) when harvested on 12–13 May but a lower ($P < 0.01$) yield (5,590 kg/ha) than TIM/360N (6,446 kg/ha) when harvested on 9–10 June.

At Cut 2, the red clover sward yielded greater ($P < 0.001$) than the other six sward species treatments (3,928 kg/ha vs. 2,406–3,293 kg/ha). IRG/360N (3,293 kg/ha) yielded greater ($P < 0.001$) than PRG/360N (2,444 kg/ha) and TIM/360N (2,406 kg/ha), while TIM/RC (3,224 kg/ha) had a greater yield than IRG/RC (2,439 kg/ha) and PRG/RC (2,638 kg/ha). In addition, the yield of IRG/360N was greater ($P < 0.001$) than that of IRG/RC, while conversely, TIM/360N had a lower yield ($P < 0.001$) than TIM/RC. There was a decline ($P < 0.05$) in Cut 2 yields as Cut 1 harvest date was delayed, with a larger decline occurring for grass/360 N than grass/RC.

At Cut 3, the red clover sward again yielded greater ($P < 0.001$) than any of the other six sward species treatments (2,675 kg/ha vs. 1,352–2,265 kg/ha) (Table 3). Among the remaining six treatments, IRG/RC (1,352 kg/ha) had a lower ($P < 0.001$) yield than PRG/RC (2,087 kg/ha) or TIM/RC (2,265 kg/ha), but there was no difference ($P > 0.05$) when the three grass species were grown with inorganic N. Grass/RC had a greater

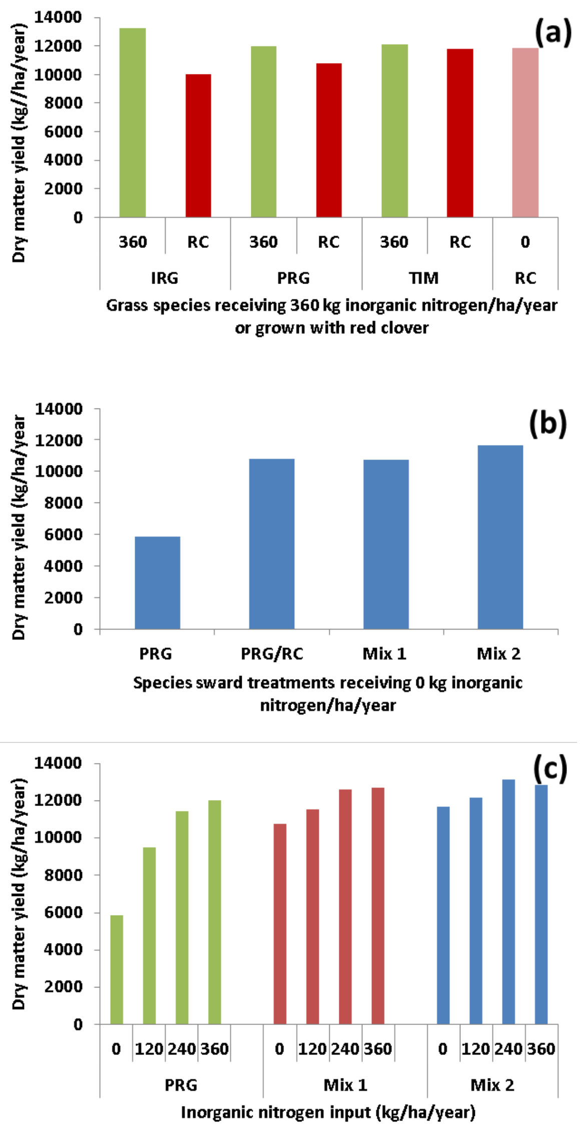

Figure 2. Annual DM yield of (a) IRG, PRG and TIM grown with 360 kg inorganic nitrogen/ha per year or with RC, and RC grown alone; (b) PRG + red clover (PRG/RC), Mix 1 and Mix 2 receiving 0 kg inorganic nitrogen/ha per year and (c) PRG, Mix 1 and Mix 2 at inorganic nitrogen rates of 0, 120, 240 and 360 kg/ha per year. All values are averaged across the Early, Middle and Late harvest schedules. DM = dry matter, IRG = Italian ryegrass, PRG = perennial ryegrass, TIM = timothy, RC = red clover.

yield than grass/360N for the Early harvest schedule (2,638 vs. 2,059 kg/ha) with the reverse outcome for the Middle (1,707 vs. 1,982 kg/ha) and Late (1,358 vs. 2,179 kg/ha) schedules, while delaying the harvest date at Cut 1 reduced (P < 0.05) Cut 3 yield only for the binary grass/clover mixtures.

At Cut 4, Italian ryegrass swards had a greater (P < 0.001) yield than timothy swards in the Early (2,000 vs. 1,485 kg/ha) and Middle harvest schedules (1,515 vs. 1,052 kg/ha) but not in the Late schedule. In general, grass/360N supported greater (P < 0.001) yields than grass/RC (1,608 vs. 1,121 kg/ha), as did (P < 0.001) the Early (1,753 kg/ha) compared to the Middle (1,265 kg/ha) and Late (1,075 kg/ha) harvest schedules.

Perennial ryegrass versus binary- and multi-species mixtures at 0N (PRG/0N, PRG/RC, Mix 1/0N and Mix 2/0N)

Annual yield

The annual DM yield of these four sward species treatments was not affected (P > 0.05) by harvest schedule. However, when averaged across the harvest schedules, annual yield was lowest (P < 0.001) for PRG/0N (5,885 kg/ha) and greatest (P < 0.01) for Mix 2/0N (1,1679 kg/ha) with PRG/RC and Mix 1/0N also yielding greater than PRG/0N (10,771 and 1,073 kg/ha, respectively) (Figure 2).

Cuts 1, 2, 3 and 4 yields

At each of Cuts 1, 2, 3 and 4, PRG/0N had the lowest (P < 0.001) yield (Table 3). Furthermore, at Cut 1, the yields of the four treatments did not differ (P > 0.05) when harvested in the Early schedule, but in the Middle schedule, the first cut yields of PRG/RC (5,120 kg/ha) and Mix 2/0N (5,558 kg/ha) were greater than that of PRG/0N (4,274 kg/ha) and the yield of Mix 2/0N was greater than that of Mix 1/0N (4,533 kg/ha). All other treatments yielded greater than PRG/0N when harvested in the Late schedule (P < 0.001) at Cut 1.

At Cut 2, the greatest (P < 0.05) yield was produced by Mix 2/0N. In addition, the Early harvest schedule (2,687 kg/ha) supported a greater (P < 0.01) yield than the Middle (2,078 kg/ha) or Late (2,031 kg/ha) schedules at Cut 2, whereas at Cut 3, the advantage for the Early schedule occurred for all sward species treatments except PRG/0N.

Perennial ryegrass versus multi-species mixtures at increasing rates of inorganic N (PRG, Mix 1 and Mix 2 at 0, 120, 240 and 360 kg N/ha per year)

Annual yield

The overall annual DM yield for PRG (9,711 kg/ha) was less (P < 0.001) than that of Mix 1 (11,893 kg/ha), which in turn was less (P < 0.05) than that of Mix 2 (12,464 kg/ha), but the scale of this average difference was greatest (P < 0.001) at 0 kg N/ha and diminished (P < 0.001) with increasing inorganic N input (Figure 2).

Cuts 1, 2, 3 and 4 yields

At each of Cuts 1–4, PRG had a lower yield than either mixture, although in some cases, this was significant only at lower rates of inorganic N input (Table 3). Thus, the yield response to inorganic N was largest and sometimes occurred only with PRG. There was a general trend for the Early harvest schedule to support lower Cut 1 yields and greater Cuts 2, 3 and 4 yields than the Middle or Late schedules, although the magnitude of these effects sometimes interacted with the rate of inorganic N input such that the differences increased in response to increasing N input at Cuts 2 and 4 but decreased in response to increasing N input at Cut 3.

Botanical composition

Mean estimates of the proportion of each species in binary- and multi-species mixtures are presented in Figure 3 (binary mixtures) and Figures 4 and 5 (multi-species mixtures).

Figure 3. Sward botanical composition – percentage of each sown species in (a) IRG/RC, (b) PRG/RC and (c) TIM/RC, at each cut in both Years 1 and 2. Values presented are the mean of values recorded by visual observations for each species at the Early, Middle and Late harvest schedules. IRG = Italian ryegrass, RC = red clover, PRG = perennial ryegrass, TIM = timothy.

Figure 4. Sward botanical composition – percentage of each sown species in Mix 1 receiving 0, 120, 240 or 360 kg N/ha per year, at each cut in both years. Values presented are the mean of values recorded by visual observations for each species at the Early, Middle and Late harvest schedules.

Figure 5. Sward botanical composition – percentage of each sown species in Mix 2 receiving 0, 120, 240 or 360 kg N/ha per year, at each cut in both years. Values presented are the mean of values recorded by visual observations for each species at the Early, Middle and Late harvest schedules.

Discussion

Perennial ryegrass, Italian ryegrass and timothy receiving inorganic N or grown with red clover (PRG/360N, IRG/360N, TIM/360N, PRG/RC, IRG/RC, TIM/RC and RC)

Grasses such as perennial ryegrass, Italian ryegrass and timothy are normally bred and evaluated under conditions of relatively high inputs of inorganic N and with some or all of the harvests being under a silage production schedule (Grogan and Gilliland, 2011). Under such conditions, the greater annual yield recorded for IRG/360N (13,229 kg/ha) than PRG/360N (11,998 kg/ha) in the current study agrees with that in Keating and O'Kiely (2000a) and Burns *et al.* (2015). That this difference arose mainly from a yield advantage at Cut 2 may be explained by Corrall *et al.* (1979) who showed that Italian ryegrass maintains its peak growth rate for a longer period in spring and summer than perennial ryegrass. Further signs of this early season growth potential of IRG/360N are illustrated by the annual yield advantage of managing it within the Early compared to the Late harvest

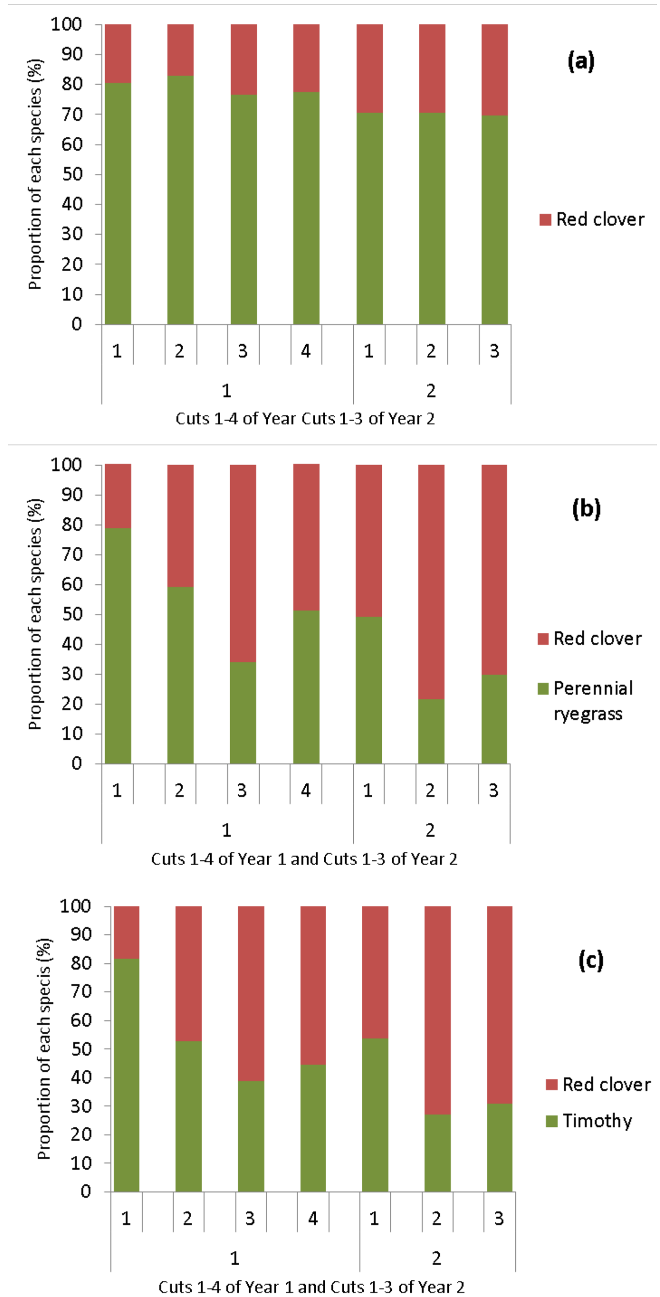

Figure 3. Sward botanical composition – percentage of each sown species in (a) IRG/RC, (b) PRG/RC and (c) TIM/RC, at each cut in both Years 1 and 2. Values presented are the mean of values recorded by visual observations for each species at the Early, Middle and Late harvest schedules. IRG = Italian ryegrass, RC = red clover, PRG = perennial ryegrass, TIM = timothy.

schedule (14,676 vs. 12,042 kg DM/ha), with the advantage of the Early schedule occurring mainly at Cut 2. The similar annual yield of PRG/360N and TIM/360N (12,097 kg/ha) agrees with that of King *et al.* (2012) who reported similar

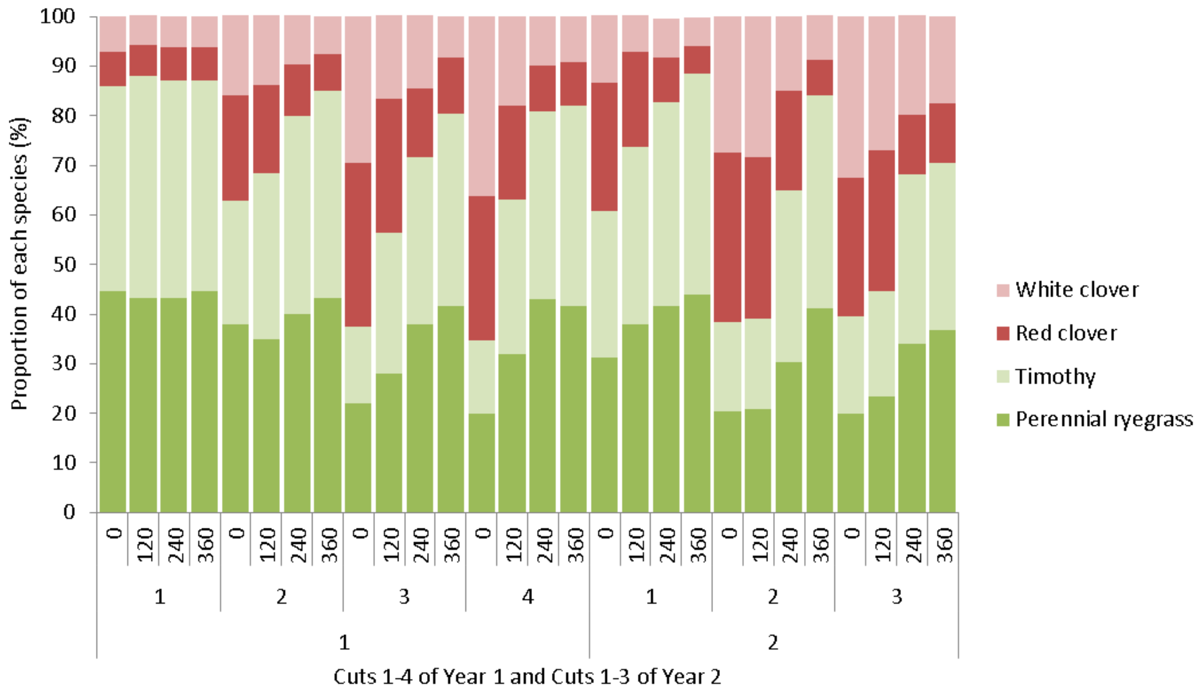

Figure 4. Sward botanical composition – percentage of each sown species in Mix 1 receiving 0, 120, 240 or 360 kg N/ha per year, at each cut in both years. Values presented are the mean of values recorded by visual observations for each species at the Early, Middle and Late harvest schedules.

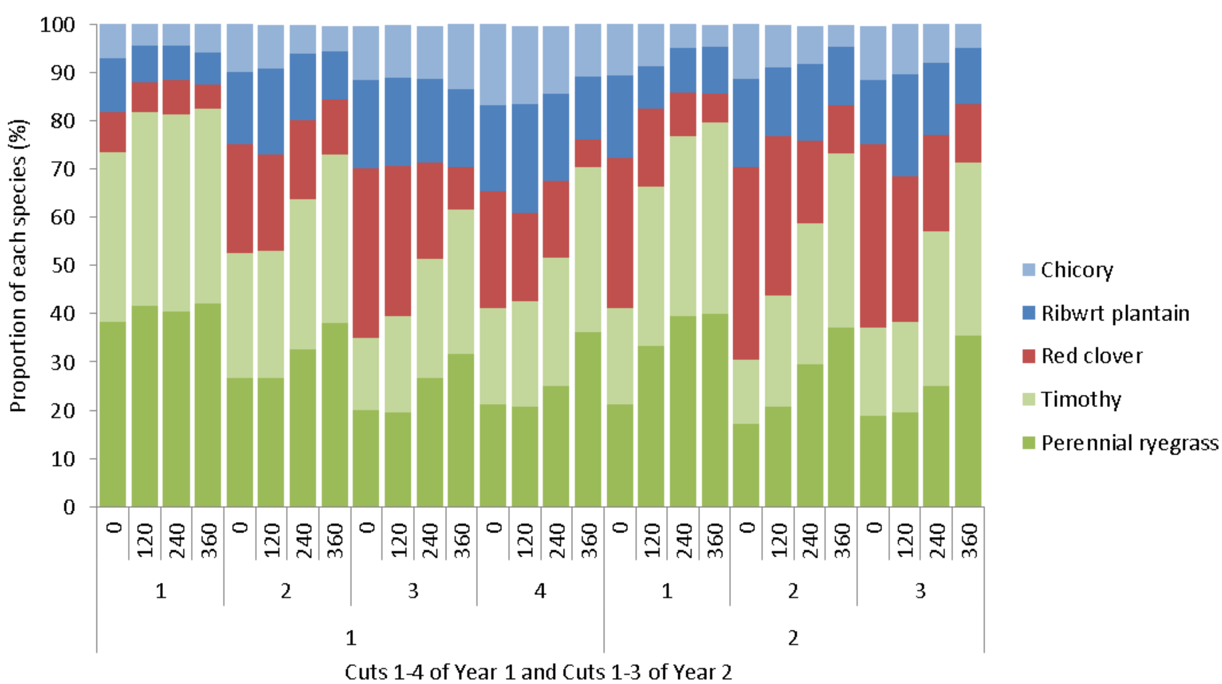

Figure 5. Sward botanical composition – percentage of each sown species in Mix 2 receiving 0, 120, 240 or 360 kg N/ha per year, at each cut in both years. Values presented are the mean of values recorded by visual observations for each species at the Early, Middle and Late harvest schedules.

yields for the primary growth harvest of perennial ryegrass and timothy at an adjacent site to the current study site.

Clavin *et al.* (2017) recorded a small increase in yield by including perennial ryegrass with red clover; however, the PRG/RC (10,771 kg/ha) binary mixture in the current study produced a lower annual yield than the red clover sward. Although both studies found a yield advantage to the binary mixture at Cuts 1 and 4, likely due to better growth of perennial ryegrass than red clover under lower temperature conditions (Lüscher *et al.*, 2005), the binary mixture suffered from a considerably greater yield reduction relative to RC at Cuts 2 and 3 in the current study. The observed reduction in yield by including perennial ryegrass with red clover may be due to the low grass proportions, particularly at Cuts 2 and 3 (Figure 3), reducing the overall N demand in the swards and thus reducing symbiotic N fixation by legumes. This negative feedback mechanism between grasses and legumes grown in mixtures has previously been reported by Nyfeler *et al.* (2011). Furthermore, the low yields of perennial ryegrass observed at Cuts 2 and 3 in the current study, even when perennial ryegrass received 360 kg N/ha per year (PRG/360N), may also be contributing to the lower than expected yields of PRG/RC. In contrast, although the proportion of red clover in the binary mixtures with perennial ryegrass or timothy was relatively similar throughout this study (Figure 3), the yield advantage of TIM/RC (11,791 and 10,771 kg/ha for TIM/RC and PRG/RC, respectively) agrees with the suggestion of Frame *et al.* (1985) that timothy and red clover can be highly successful sward companions.

The yield advantage of Italian ryegrass over perennial ryegrass when supplied with 360 kg inorganic N/ha per year disappeared when they were instead grown with red clover. This change probably reflects the considerably lower red clover content in the sward for IRG/RC than for PRG/RC, particularly during mid-season (Figure 3), which was likely a result of the more vigorous growth of Italian ryegrass early in the season (Ledgard and Steele, 1992). The lower red clover content would have meant that firstly the direct yield contribution of red clover would have been reduced at a stage of the season when it exhibited a greater growth rate than that of Italian or perennial ryegrass and secondly that provision of fixed N to its companion grass would likely have been reduced (Elgersma *et al.*, 2000). Kunelius and Narasimhalu (1983) concluded that high yields could be achieved from mixtures of Italian ryegrass and red clover provided the seed rate and row spacing favoured the establishment and persistence of the legume component.

These changes in annual herbage yields between Italian ryegrass, perennial ryegrass and timothy, depending on whether they are grown as monocultures and have inorganic N applied or are part of a binary mixture with red clover, indicate that yield differences between species grown as monocultures cannot be assumed to hold true when these species are included in the binary mixture and probably also in more complex species mixtures.

The mean growth rates from 12–13 May (Early harvest schedule) to 9–10 June (Late harvest schedule) of the primary growth of the IRG/360N, PRG/360N and TIM/360N monocultures (3, 19 and 24 kg DM/ha per day, respectively) were considerably below the corresponding values of 140, 113 and 175 kg DM/ha per day reported by King *et al.* (2012) for these species with the reasons for the low growth rates in the current study unclear. This is in contrast to monocultures of red clover where the growth rates (80 kg DM/ha/day) were the same in both the current study and King *et al.* (2012). The finding, therefore, that a binary mixture of Italian ryegrass with red clover exhibited a similarly low growth rate to IRG/360N over this critical 28-day period of the primary growth (9 kg DM/ha/day) whereas perennial ryegrass or timothy in combination with red clover displayed greatly improved growth rates (78 and 76 kg DM/ha per day, respectively) suggests that, at comparable seeding rates to this study, Italian ryegrass may not have a worthwhile role in multi-species grassland swards managed for silage production when red clover is an important component of the legume functional group. This suggestion is supported by the conclusions of Kunelius and Narasimhalu (1983).

Perennial ryegrass versus binary- and multi-species mixtures at 0N (PRG/0N, PRG/RC, Mix 1/0N and Mix 2/0N)

The average annual DM yield of 5,862 kg/ha recorded for PRG/0N is 55% and 94% of the mean values reported by Conaghan *et al.* (2012) and Keating and O'Kiely (2000b), respectively, under comparable conditions of silage production management and 0 kg inorganic N input/ha per year, with 67% of the annual yield harvested at Cut 1 in the current study. The inclusion of red clover in a binary mixture with perennial ryegrass increased annual yield by 83%. This increase was distributed across all cuts but occurred in particular at Cuts 2 and 3 when the red clover proportion was likely at its most productive (Elgersma *et al.*, 2000). It is noteworthy that at Cut 1, the binary mixture outyielded both of its constituent species grown in single-species swards, whereas at Cuts 2 and 3, the binary mixture yield was intermediate between its constituent single-species swards. This trend was also reported by Clavin *et al.* (2017). This suggests that at Cut 1, and also at Cut 4, synergy between the grass and legume resulted in either or both species performing considerably better in binary mixture than when grown alone. Such trends may be due to the negative feedback mechanisms associated with the grass and clover proportions (Figure 3) described by Nyfeler *et al.* (2011), which have been discussed previously.

The relatively similar yields of PRG/RC and Mix 1/0N annually (10,771 and 10,738 kg/ha, respectively) and at each cut suggests that much of the yield advantage of Mix 1/0N over PRG/0N is attributable to the legumes present and that the grass and legume functional groups performed as well in PRG/RC as in Mix 1/0N. This is supported by Spehn *et al.* (2002) who identified legumes, and specifically clover species, as key drivers of productivity in multi-species swards. The reduction in the red clover content in Mix 1/0N compared to that in PRG/RC (Figures 3 and 4) without a negative effect on treatment yield and with only relatively modest effects on the proportion of legume present suggests that white clover largely compensated for the reduced content of red clover both in terms of legume yield and the amount of N fixed (Black *et al.*, 2009). Although the design of the current study does not enable a conclusion be drawn on the relative effects of red and white clovers on N fixation within Mix 1/0N, the findings of Clavin *et al.* (2017) and Kirwan *et al.* (2007) suggest that over the range of red clover contents present in PRG/RC and Mix 1/0N that red clover could fix sufficient atmospheric N to support productive growth of grass.

The 9% increase in annual DM yield for Mix 2/0N (11,679 kg/ha) over Mix 1/0N (10,738 kg/ha) likely reflects the increase in plant functional diversity by replacing white clover with ribwort plantain and chicory in MIX 2/0N. The production benefits of adding ribwort plantain and/or chicory to grass-clover swards have also been demonstrated by Cong *et al.* (2018), while Pembleton *et al.* (2015) and Hector *et al.* (1999) concluded that increasing functional rather than species diversity had a greater influence on sward productivity. It would, however, be important that there is sufficient actively N-fixing red clover present to support the non-N-fixing species in the sward. Thus, the modest yield increase from Mix 1/0N to Mix 2/0N coupled with the generally quite similar contents of red clover in both mixtures suggests that red clover in this instance was enabling elevated herbage growth in these mixtures, and this agrees with the conclusions of Spehn *et al.* (2002).

The considerable benefit of complementing PRG/0N with a productive species from the legume functional group either as part of a binary- or a multi-species mixture is evident from the growth rates of these herbages (PRG/RC, Mix 1/0N and Mix 2/0N) during the interval between 12–13 May and 9–10 June when DM yields under silage production management are expected to increase rapidly. Therefore, despite all four treatments having generally similar yields on 12–13 May, the markedly greater subsequent daily growth rates for PRG/RC (78 kg DM/ha), Mix 1/0N (64 kg DM/ha) and Mix 2/0N (79 kg DM/ha) compared to those for PRG/0N (8 kg DM/ha) indicate the commencement of an advantage that then continued through Cuts 2 and 3, as previously discussed.

Perennial ryegrass versus multi-species mixtures at increasing rates of inorganic N (PRG, Mix 1 and Mix 2 at 0, 120, 240 and 360 kg N/ha per year)

The increases in the annual DM yield of perennial ryegrass of 61%, 94% and 104% in response to the application of 120, 240 and 360 kg inorganic N/ha per year, respectively, agree with those in Keating and O'Kiely (2000b) and Conaghan *et al.* (2012) who reported a positive yield response at up to 430 kg N/ha per year. Compared to the proportionate responses recorded in the annual DM yield of PRG to the three incremental rates of inorganic N, much smaller corresponding responses of 7%, 17% and 18%, and 12% and 10% occurred for Mix 1 and 4, and Mix 2, respectively (Table 5). These relatively poor responses to applied N may be due to a combination of factors (Whitehead, 1995). First, high rates of symbiotic N fixation by the legumes within Mix 1 and Mix 2 could provide the plants in these treatments with sufficient N to then reduce their subsequent response to additional N provided in the inorganic form, as concluded by Nyfeler *et al.* (2009). Second, since an effect of inorganic N application was to reduce the amount of legume present in the sward (Figures 4 and 5) and possibly also its rate of N fixation (Hartwig, 1998; Nyfeler *et al.*, 2011), then some of the inorganic N applied would effectively have partially replaced N that would have been fixed by a greater content or activity of legume in the sward. Thus, inorganic N may have compensated for the reduction in biologically fixed N rather than make a net positive contribution to the pool of available N, leading to a reduced yield response (Craven *et al.*, 2016). Furthermore, the largest response of both Mix 1 and Mix 2 to applied N occurred early in the season at Cut 1 and with a minimal response at subsequent cuts. This greater response to inorganic N early in the season is likely due to a combination of rapid grass growth rates when in the inflorescence physiological phase (Brereton, 1992; Whitehead, 1995) and restricted legume growth and biological N fixation due to cooler ambient temperatures during the growth prior to Cut 1 (Liu *et al.*, 2011; Phelan *et al.*, 2015).

The suggestion of Hopkins and Holz (2006) that multi-species swards can express an annual DM yield advantage over perennial ryegrass monocultures when both sward types receive not more than 150 kg inorganic N/ha per year is supported by the results from the current study. Furthermore, the finding that Mix 1 or Mix 2 managed at 0 and 120 kg N/ha per year produced yields comparable to perennial ryegrass receiving 240 and 360 kg inorganic N/ha per year agrees with that of Nyfeler *et al.* (2009) who recorded comparable yields between a perennial ryegrass monoculture receiving 450 kg N/ha per year and a four-species grass–legume mixture receiving 50 kg N/ha per year. The latter authors attributed the yield advantage of the multi-species sward to the contribution of its high legume proportion. Furthermore, the higher rates of inorganic N application would have led to the inhibition of symbiotic fixation of N_2 by legumes as reported by Nyfeler *et al.* (2011).

In the current study however, despite a large reduction in the legume content due to inorganic N application (Figures 4 and

Table 5. Yield response[1] of PRG, Mix 1 and Mix 2 to each increment of inorganic N (kg additional herbage DM/kg inorganic N applied)

Increment	0–120 kg N/ha per year			120–240 kg N/ha per year			240–360 kg N/ha per year		
Species	PRG	Mix 1	Mix 2	PRG	Mix 1	Mix 2	PRG	Mix 1	Mix 2
Cut 1	34	21	11	9	8	13	9	2	–5
Cut 2	31	–4	–3	18	10	6	0	–2	–4
Cut 3	27	–3	–4	23	8	7	2	1	0
Cut 4	27	9	12	18	10	3	8	1	4
Annual	30	7	4	16	9	8	5	1	–2

[1]Yield response equals the increase in herbage DM yield for each increment of inorganic nitrogen applied.
PRG = perennial ryegrass, DM = dry matter.

5), Mix 1 and Mix 2 produced greater yields at individual cuts and annually than PRG receiving high rates of inorganic N. This suggests that even at low proportions in the sward, and under conditions of high inorganic N input, legumes made a measurable contribution to the overall DM yield. This agrees with Kirwan *et al.* (2007) who reported the productivity of grass–legume mixtures to be robust over a wide range of legume proportions, while Connolly *et al.* (2009) reported that a positive species mixture effect could still persist for a time even after the visual disappearance of legumes from mixed-species swards.

Herbage growth rates around the time when the primary growth of grassland swards is harvested for silage production are frequently among the highest recorded during the year (Brereton, 1992). This can encourage farmers to defer harvesting in order to quickly boost the yields ensiled. However, the disappointing growth rates of perennial ryegrass swards during this stage of their primary growth in the current study suggest that minimal yield advantage would have accrued from further deferring perennial ryegrass harvest date. Considering that (a) perennial ryegrass growth rates between 12–13 May and 9–10 June showed little response to progressively greater rates of inorganic N application in mid-March and (b) the growth rates achieved were generally lower than those simultaneously exhibited by Mix 1 and Mix 2 at comparable inorganic N inputs, an important possibility is that the yield response of perennial ryegrass swards to inorganic N was largely completed by 12–13 May under the conditions prevailing and that applying the full primary growth allocation of inorganic N in mid-March might not have been optimal. Furthermore, this N may have been lost by leaching or as greenhouse gases. In contrast, the greater growth rates by Mix 1 and Mix 2 might be at least partially a response to a more ongoing supply of N made available by *Rhizobia* bacteria in legume root nodules. The rate of this ongoing supply would likely have adjusted according to the demands of the sward (Nyfeler *et al.*, 2011), possibly resulting in greater use efficiency.

Carryover effects of harvest schedule

Gilliland *et al.* (1995) reported that delaying the first harvest of a perennial ryegrass sward in order to increase DM yield led to relatively lower yields harvested at a subsequent second harvest after 7 weeks regrowth. This was likely due to self-thinning of grass tillers in response to poor light penetration in heavier yielding swards, which meant fewer tillers in the regrowth (Davies and Simons, 1979). A similar phenomenon was recorded in the current study for single-, binary- and multi-species swards. Note however that the magnitude of the carryover effect of the date of first harvest was smaller for binary grass–legume than single-species grass swards. This suggests that the presence of red clover in the binary mixtures compensated for some of the tiller thinning that likely occurred in the grass component of these swards. Furthermore, and similar to the results of Gilliland *et al.* (1995), any carryover effects on subsequent harvests were minimal or absent.

Botanical composition

Sward persistence is an economically important trait on grassland farms (O'Donovan *et al.*, 2017), and multi-species swards need to exhibit persistence under on-farm conditions if they are to be used by farmers. The current study assessed sward botanical composition only during the second and third years after sowing, and this is over a considerably shorter duration than optimal to make a secure assessment of persistence. However, the relatively high content of legumes in Mix 1/0N and of legume plus herbs in Mix 2/0N throughout this time period, and with no indications of a temporal decline in their contents or herbage yield relative to grass swards receiving high rates of N, suggests that these two multi-species swards could persist in a silage production system in the absence of inorganic N input (Figures 4 and 5). The apparent negative impact of inorganic N application on the presence of both the legume and herb functional groups suggests that the use of mixtures such as Mix 1 and Mix 2 may not be viable under conditions of elevated inorganic N input without adaptive management. Previous studies have demonstrated the benefits of multi-species swards despite fluctuating species proportions under high N input systems (Nyfeler *et al.*, 2009; Finn *et al.*, 2013; Enriquez-Hidalgo *et al.*, 2016); however, as is the case in the current study, the number of experimental years may not have been sufficient to fully assess the long-term effects of intensive agricultural management practices on the persistence of such multi-species mixtures (Sturludóttir *et al.*, 2014). Future research needs to make such assessments over considerably longer time frames and differing farm conditions. Furthermore, it will also need to determine sward persistence following the provision of N via slurry application, as necessarily occurs on many grassland farms. These assessments need to be made using similar machinery systems as operated on commercial farms.

Conclusion

The different annual yield ranking of the three grass species when receiving 360 kg inorganic N/ha per year (Italian ryegrass > perennial ryegrass = timothy) compared to the sward yield ranking when each was in binary mixture with red clover (Italian ryegrass = perennial ryegrass < timothy) indicates that it is not appropriate to assume that the yield of a binary mixture will be a product of its component species grown separately.

The magnitude of annual yield increases from perennial ryegrass managed without inorganic N input (PRG/0N) to PRG/RC, Mix 1/0N and Mix 2/0N suggests that much of the yield advantage of the binary- and multi-species mixtures derived from the inclusion of N-fixing legumes (red and white clovers) and with a small further benefit accruing from replacing white clover with complementary species from a third functional group (the herbs ribwort plantain and chicory). In addition, within the limited time frame available to assess sward persistence, both Mix 1 and Mix 2 appeared capable of persisting when the management regime operated without inorganic N being applied.

The multi-species treatments Mix 1 and Mix 2 provided an annual yield advantage over perennial ryegrass (PRG) under the prevailing conditions, and even though this was evident at each rate of N input, it was particularly so at zero or low input rates. Accordingly, Mix 2/0N and both Mix 1/120N and Mix 2/120N matched the annual yield achieved by PRG/360, and this demonstrates an opportunity to reduce inorganic N input without compromising annual yield in grassland systems. Reasons not to apply high rates of inorganic N to multi-species swards such as Mix 1 and Mix 2 include (1) their generally low DM yield response to applied inorganic N, which would be expected to have negative economic and environmental implications, (2) the likely resultant low recovery rate of such applied N within harvested herbage, with the implication of the inferred losses of N causing follow-on negative environmental consequences and (3) the probable negative impact on sward persistence, albeit this was gleaned from a limited time frame. Future research, however, could usefully assess if moderate rates of inorganic N applied only to stimulate the primary growth in spring might be worthwhile.

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